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Preparation for emergence of an Eastern European porcine reproductive and respiratory syndrome virus (PRRSV) strain in Western Europe: Immunization with modified live virus vaccines or a field strain confers partial protection

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ABSTRACT

The porcine reproductive and respiratory syndrome virus (PRRSV) causes huge economic losses for the swine industry worldwide. In the past several years, highly pathogenic strains that lead to even greater losses have emerged. For the Western European swine industry, one threat is the possible introduction of Eastern European PRRSV strains (example Lena genotype 1.3) which were shown to be more virulent than common Western resident strains under experimental conditions. To prepare for the possible emergence of this strain in Western Europe, we immunized piglets with a Western European PRRSV field strain (Finistere: Fini, genotype 1.1), a new genotype 1 commercial modified live virus (MLV) vaccine (MLV1) or a genotype 2 commercial MLV vaccine (MLV2) to evaluate and compare the level of protection that these strains conferred upon challenge with the Lena strain 4 weeks later. Results show that immunization with Fini, MLV1 or MLV2 strains shortened the Lena-induced hyperthermia. In the Fini group, a positive effect was also demonstrated in growth performance. The level of Lena viremia was reduced for all immunized groups (significantly so for Fini and MLV2). This reduction in Lena viremia was correlated with the level of Lena-specific IFNγ-secreting cells. In conclusion, we showed that a commercial MLV vaccine of genotype 1 or 2, as well as a field strain of genotype 1.1 may provide partial clinical and virological protection upon challenge with the Lena strain. The cross-protection induced by these immunizing strains was not related with the level of genetic similarity to the Lena strain. The slightly higher level of protection established with the field strain is attributed to a better cell-mediated immune response.

1. Introduction

Since the emergence of the porcine reproductive and respiratory syndrome virus (PRRSV) almost 30 years ago, the disease has spread to most swine-producing countries around the world. PRRSV infection is mainly characterized by reproductive disorders in sows and respiratory syndrome and growth retardation in growing pigs (Albina, 1997). Due to the direct and indirect costs of the disease, PRRS is recognized as one of the most important economic diseases for the swine industry worldwide (Neumann et al., 2005). PRRS also must be considered in terms of public health, because studies have shown that PRRSV infection may facilitate the spread or the maintenance of zoonotic bacteria or viruses in pigs, such as *Salmonella* or the hepatitis E virus (Beloeil et al., 2004; Salines et al., 2015).

Historically, two different PRRS viruses emerged nearly at the same time in the late 1980s; genotype 1 in Europe and genotype 2 in North America and Asia. Additional genetic analyses can distinguish between different subtypes among genotype 1 strains (Stadejek et al., 2013). Subtype 1 strains — mainly circulating in Western Europe but also present in Asia and North America — are considered to be predominantly low pathogenic strains, whereas subtype 3 strains circulating in Eastern Europe are considered to be more virulent (Morgan et al.,

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2013). Lena strain is a prototype subtype 3 strain which has been extensively studied over the past few years (Karniychuk et al., 2010; Renson et al., 2017; Trus et al., 2016; Weesendorp et al., 2013). This strain was isolated in 2007 in a Belarusian farm where severe reproductive failure and high mortality rate in growing pigs were reported (Karniychuk et al., 2012).

Due to the growing economic exchanges between Western and Eastern Europe, and in particular the flow of live pigs and trucks circulating between these two parts of Europe, the risk of spread of a subtype 3 PRRSV strain from Eastern to Western Europe is high. Considering (i) the virulence these strains had demonstrated under numerous experimental conditions (Karniychuk et al., 2010; Morgan et al., 2013; Renson et al., 2017; Weesendorp et al., 2014), and (ii) the naïve immune status of the Western Europe pig populations regarding subtypes 3 strains (Stadejek et al., 2008), their introduction in the Western European pig industry would certainly have a huge economic impact.

Preparation for the possible emergence of such strains in Western Europe calls for an investigation on the protection provided by previous immunization of pigs with field or vaccine strains. Regarding vaccines, two studies have already explored the protection afforded by a modified live virus (MLV) vaccine of genotype 1 upon challenge with the Lena strain in growing pigs. Both studies showed partial clinical and virological protection in vaccinated pigs (Bonckaert et al., 2016; Trus et al., 2014). Concerning PRRSV field strains, only one study has explored the heterologous protection provided by genotype 1.1 Belgian PRRSV strains (Trus et al., 2016). Here also, the obtained protection was partial. In contrast, a recent study demonstrated that immunization with the Lena strain provides a complete protection against a homologous challenge (Weesendorp et al., 2016).

Although these previous studies have provided very interesting data, they also have some caveats because they used MLV vaccines developed from old PRRSV strains and they did not evaluate MLV vaccines of genotype 2 which are now licensed for use in many Western European countries. Another limitation is that the protection provided by PRRSV vaccine and field strains was not investigated simultaneously, hindering comparison of the protection level conferred by attenuated or non-attenuated PRRSV strains.

Thus, considering these remaining questions, the objective of the present study was to evaluate and compare the protection provided by the immunization with a Western European field (genotype 1.1) PRRSV strain, a new genotype 1 MLV vaccine or a genotype 2 MLV vaccine upon challenge with the genotype 1.3 Lena strain in growing pigs.

2. Materials and methods

2.1. Vaccine and virus strains

The genotype 1 Ingelvac PRRSFLEX® EU vaccine (Boehringer Ingelheim France, Paris, France, 94881 strain, GenBank accession no. KT988004) and the genotype 2 Ingelvac® PRRS MLV vaccine (SCS Boehringer Ingelheim Comm, Brussels, Belgium, USA ATCC VR2332 strain, GenBank accession no. EF484033) were used in the in vivo experiment as MLV1 and MLV2 vaccines, respectively. For in vitro (enzyme-linked immunospot) ELISPOT analyses, MLV1 and MLV2 vaccine strains were obtained by suspending the respective lyophilized Ingelvac vaccines in Eagle's minimal essential medium (EMEM), propagating them once and titrating them on MARC145 cells.

The genotype 1.1 Finistere PRRSV strain (PRRS-FR-2005-29-24-1) was isolated in France in 2005 from a herd with reproductive failures in sows (abortions). In specific pathogen-free (SPF) pigs, Finistere infection induces a mild clinical expression (Rose et al., 2015). The genotype 1.3 Lena PRRSV strain (GenBank accession no. JF802085) was kindly provided by Dr. Hans Nauwynck (University of Ghent, Belgium). The Lena strain was isolated in Belarus in 2007 from a herd with mortality, reproductive failures and respiratory disorders (Karniychuk et al., 2010). The Finistere and the Lena strains were propagated and titrated on pulmonary alveolar macrophages for 2 and 5 passages, respectively, for animal inoculations, and for 4 and 7 passages, respectively, for ELISPOT analyses. For virus neutralizing tests, a Lena strain adapted to MARC145 cell culture was kindly provided by Dr. Hans Nauwynck (University of Ghent, Belgium), then propagated for 5 passages and titrated on MARC145 cells.

2.2. Finistere strain full genome sequencing

The full genome sequence of the PRRSV Finistere strain was obtained using next-generation sequencing (NGS). Viral RNA purification, cDNA synthesis and library construction was prepared as described in (Brown et al., 2016). NGS was done at the Biogenouest (Nantes, France) core facility using a MiSeq HD Sequencer (Illumina). Then, bioinformatic reconstruction of the full-length genome was performed by cleaning the sample reads using Trimmomatic software (Bolger et al., 2014). A Bowtie2 2.1.0 (Langmead and Salzberg, 2012) alignment on the *Sus scrofa* genome eliminated most of the host reads. Remaining reads were compared against the ViPR database (Pickett et al., 2012) to extract the viral sequences. An accurate alignment afforded a first set of viral reads. These reads did not cover nucleotides 2310 to 2603 in the reference Lelystad strain full genome (GenBank accession no. M96262). A tblastn 2.2.28 (Camacho et al., 2009) on the related amino acid sequence afforded the determination of a second set of reads matching this area in our sample. Both sets were submitted to kmergenie 1.5658 (Chikhi and Medvedev, 2014) and vicuna 1.3 (Yang et al., 2012), providing us the first draft genome of Finistere strain as a single contig. The Finistere PRRSV strain sequence was deposited in GenBank under accession no. KY366411.

2.3. Vaccine and virus sequence comparison

Comparison of the genome of the immunizing strains with that of the Lena strain was performed using the Simplot 3.5.1 program (Lole et al., 1999) which plots similarity versus position based on the percentage of identity obtained by alignment of the full-genome sequences using the MUSCLE algorithm (Edgar, 2004).

2.4. Experimental setting

Forty-one, four-week-old pure Large White piglets coming from a nucleus herd (free of PRRSV, *Actinobacillus pleuropneumoniae, Mycoplasma hyopneumoniae*) were housed in our biosecurity level-3 air-filtered animal facilities. The 41 piglets were randomly assigned to five groups housed in separate rooms (Table 1). At 6 weeks of age (D-27), 7 piglets were inoculated intranasally with the PRRSV Finistere strain (5×10^5 50% tissue culture infectious dose (TCID50) per piglet) (Fini group). At the same time, 9 piglets were vaccinated intramuscularly with either the MLV1 (minimum dose $10^{4.4}$ TCID50/piglet) or the MLV2 vaccine (minimum dose $10^{4.9}$ TCID50/piglet). At 10 weeks of age (D0), all the piglets from the Fini, MLV1 and MLV2 groups were challenged intranasally with the Lena strain (genotype 1.3, 5×10^5

Table 1
Experimental design.

	Immunization	Challenge
Group	(D −27)	(D0)
Control (n = 8)	–	–
Lena (n = 8)	–	Lena (genotype 1.3, IN)
Fini (n = 7)	Finistere strain (genotype 1.1, IN)	Lena (genotype 1.3, IN)
MLV1 (n = 9)	Ingelvac PRRSFLEX EU (genotype 1.1, IM)	Lena (genotype 1.3, IN)
MLV2 (n = 9)	Ingelvac PRRS MLV (genotype 2, IM)	Lena (genotype 1.3, IN)

IN: intranasal; IM: intramuscular.